A4.4:180

Issued July 22, 1911.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—CIRCULAR 180.

A. D. MELVIN, CHIEF OF BUREAU.

A METHOD FOR THE SEPARATION OF THE SEVEN PERMITTED COAL-TAR COLORS WHEN OCCURRING IN MIXTURES.

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INTRODUCTION.

The regulations of the Secretary of Agriculture governing meat inspection allow the use of certain dyes on casings of sausages and other meat food products when the character of the casing is such that the dye will not penetrate into the meat food product contained therein. The coal-tar dyes permitted to be used for this purpose are as follows:

Red shades:
107. Amaranth.
56. Ponceau 3 R.
517. Erythrosin.
Orange shades:
85. Orange 1.
Yellow shades:
4. Naphthol Yellow S.
Green shades:
435. Light Green S F Yellowish.
Blue shades:
692. Indigo Disulfo Acid.

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The numbers preceding the names refer to the numbers of the dyes as listed by Green.[1]

To obtain desired shades meat packers often employ a mixture of dyes, and in such cases it becomes necessary to determine what colors are contained in the mixture. There is little in the literature where the question of separating mixtures of colors is considered. The chief object of most work has been to obtain methods for identifying colors when they occur in a pure form.

Rota[2], who has a most excellent scheme for recognizing pure colors by their chemical reactions, makes the following statement in regard to the separation of coloring matters in a mixture:

> It is sometimes possible to effect the separation by treating the mixture with water or alcohol at the ordinary temperature or with the aid of heat, but as a rule extract-

[1] A. G. Green. Schultz and Julius Systematic Survey of the Organic Coloring Matters. 1904.
[2] A. R. Rota. Chemiker Zeitung, 1898, pp. 437–442.

ing with ether or by fixing the dye on wool or other fiber is the most promising method.

Green[3], assisted by Yoeman and Jones, gives a valuable means of identifying pure colors by applying the dyestuff to animal fiber. Under the head of "Mixtures" these authors state:

It is not our intention in this paper to deal fully with the detection of mixtures. A few general principles may be mentioned here which will be found useful by those who wish to extend the scheme to such cases. If a mixture consists of two or more dyestuffs of the same chemical group, it will behave as a whole similarly to a single dyestuff, though sufficient differences will usually exist in the rate of solution or of attack by the group reagent to render it possible to distinguish or even separate the constituents.

Mulliken[4], in discussing the identification of dyestuffs in mixtures, states that infallible rules for the isolation of individual dyes from all classes of mixtures can not be formulated. He gives, however, suggestions for analytical procedures which may be applied in certain cases. He closes his chapter on "The identification of dyestuffs in mixtures" with the following remarks:

In official laboratories where the chief concern is to determine whether colors under examination belong to a certain limited list of legally permitted dyestuffs, carefully prepared standard wool, silk, and cotton dyeings and samples of their after chromed wool dyeings should prove very helpful for purposes of comparison. One of the most valuable auxiliaries in this class of work is, however, the spectroscope. This is well illustrated by the case of the seven colors permitted in foods under the regulations of the American Federal pure-food law. Six of these colors have excellent absorption spectra. Some of the bands, it is true, overlap and interfere at certain concentrations in certain mixtures, but any of them may be identified in the presence of the others if a partial separation is first effected by the fractional dyeing or stripping procedure. One of them—Indigo Disulfo Acid—which, contrary to the general rule, is rather rapidly destroyed by heating with ammonia, can not be satisfactorily identified when present in mixtures in small proportions by the fractional stripping and dyeing processes and may therefore be easily overlooked; but it is easily detected by the spectroscope. Naphthol Yellow S, on the other hand, having no characteristic spectrum, can only be separated by fractional methods and identified by chemical tests. Hence it may easily escape observation in complex mixtures when present in small quantities, its complete separation by fractional stripping and dyeing from Naphthol Orange A being slow and difficult.

With the exception of this statement by Mulliken, the writer has been unable to find any mention in the literature of a process for the separation of the seven permitted coal-tar colors when they occur in mixtures. For a method to be of value for this purpose, it should not only enable the analyst to separate these colors so that each color may then be identified, but at the same time it should enable him to detect the presence of any prohibited color which may occur in the mixture. The identification of such prohibited color would not neces-

[3] A. G. Green. Journal of the Society of Dyers and Colourists, September, 1905, p. 236.
[4] Mulliken. Identification of Pure Organic Compounds. Commercial Dyestuffs, vol. 3, 1910.

sarily be essential, for its mere presence would be sufficient to exclude the mixture from use for coloring the casings of inspected meat-food products.

THE AUTHOR'S EXPERIMENTS AND METHOD.

The first work toward outlining a method for this separation was to make a thorough study of the solubility of the seven permitted colors in various solvents and also to determine the precipitants for each. This was followed by determining what solvents would remove the colors by agitation from acid, alkaline, and neutral solutions. After this preliminary work had been conducted, known mixtures of the seven colors were taken and attempts were made to separate each color by applying the information gained through the preliminary work. The following method was finally adopted and has proved to be a valuable means of separating the seven permitted coal-tar colors when they occur in mixtures:

REAGENTS.

It is of the utmost importance that the reagents be employed of the strength indicated below:

Ammonium sulphate solution.—A supersaturated solution of ammonium sulphate is prepared by agitating ammonium sulphate in water at steam bath temperature until the water is completely saturated. This will require several hours. The solution is then allowed to cool at room temperature and should always contain an excess of undissolved ammonium sulphate crystals. The solution should always be agitated previous to using.

Sodium chlorid solution.—A supersaturated solution of sodium chlorid is prepared in the same manner as the supersaturated ammonium sulphate solution described above. The solution should stand in contact with undissolved crystals of the salt.

Calcium chlorid solution (25 per cent).

25 grams of calcium chlorid, C. P. granulated, in
100 c. c. of water.

Dilute ammonia (1:100).

1 c. c. of concentrated ammonia in
100 c. c. of water.

Dilute acetic acid (5 per cent).

5 c. c. glacial acetic acid.
95 c. c. of water.

Stannous chlorid solution.—Dissolve 30 grams of granulated tin, free from iron, in 125 c. c. of hydrochloric acid (1.19 specific gravity); dilute to 250 c. c. and filter through asbestos. To the filtrate add 250 c. c. of hydrochloric acid (1.124 specific gravity) and 500 c. c. of water.

Acetone.
Alcohol (*95 per cent*).
Amyl alcohol.
Acetic ether (*ethyl acetate*).
Ether, sulphuric (*ethyl ether*).
Hydrogen peroxid (*3 per cent*).

PROCEDURE.

PRELIMINARY TEST.

The well-known method of scattering the powdered dyestuff upon water, alcohol, and sulphuric acid should be used to determine whether one or more colors are present.

METHOD OF SEPARATION OF THE SEVEN PERMITTED COLORS.

Approximately 0.2 of a gram of the sample is rubbed up in a mortar with about 25 c. c. of supersaturated ammonium sulphate solution, filtered, and if the filtrate is colored red the residue on the filter is washed with small portions of the supersaturated ammonium sulphate solution until the washings are no longer colored red. The filtrate and washings should contain Amaranth, together with some Naphthol Yellow S. The filtrate and washings are combined and shaken with separate portions of acetic ether until the acetic ether is no longer colored yellow. The acetic ether removes the Naphthol Yellow S, and the Amaranth remains behind in the ammonium sulphate solution. The acetic ether extracts containing the Naphthol Yellow S need not be preserved, as this color will be removed in a pure form later. The ammonium sulphate solution containing the Amaranth is shaken with acetone, which extracts the Amaranth. The acetone is then filtered to remove the excess of salt, diluted with a small amount of water, and the acetone driven off on the steam bath. The water solution is then tested for Amaranth.[1]

The undissolved portion of the original sample is removed from the filter, mortar, and pestle by washing with water. This water solution is acidified with acetic acid and shaken with separate portions of ether until the ether is no longer colored. The ether will remove the Erythrosin. It is washed several times with water and finally shaken up with dilute ammonia solution. The Erythrosin is taken up by the ammonia solution. The excess of ammonia is driven off on the steam bath and the solution tested for Erythrosin. Observation should be carefully made to see that this solution when very dilute has no fluor-

[1] For the identification of all the separated colors the reactions as given by Loomis (Circular 63, Bureau of Chemistry, U. S. Department of Agriculture, 1911) should be used.

escence, as this property indicates the presence of prohibited colors having similar reactions.

The water solution which has been extracted with ether is warmed on the steam bath until all the ether is removed. It is then saturated at the steam bath temperature with sodium chlorid, cooled, and filtered through a folded filter paper 18 c. m. in diameter. This filtration should be carried out so as to keep the precipitate in the bottom of the filter. The precipitate is washed with supersaturated sodium chlorid solution until the washings are colorless. The filtrate and washings should contain Light Green S F Yellowish, Naphthol Yellow S, and possibly traces of Orange 1. They are combined and shaken with acetone, which removes the Naphthol Yellow S. This acetone solution should be washed several times by agitation with supersaturated sodium chlorid solution in order to remove any traces of green color held mechanically by the acetone. A small amount of water is added and the acetone driven off on the steam bath. The water solution is acidified with acetic acid and shaken with amyl alcohol, which removes any traces of Orange 1 that may be present. The water solution is freed from amyl alcohol by heating on the steam bath and is then tested for Naphthol Yellow S.

The solution containing Light Green S F Yellowish is evaporated to a small bulk and the residue is taken up in alcohol and filtered. This removes excess of salt. The alcohol is driven off and the solution then tested for Light Green S F Yellowish.

The precipitate on the filter that has been well washed with the supersaturated sodium chlorid solution should contain Orange 1, Ponceau 3 R, and Indigo Disulfo Acid. This precipitate is dissolved in water and shaken with several successive portions of acetic ether. Orange 1 is taken up by the acetic ether. It is not necessary, however, to continue this extraction with acetic ether until all of the Orange 1 is removed. About three extractions will remove enough Orange 1 to make all the necessary tests for this color. The acetic ether portions are combined and washed with supersaturated sodium chlorid solution several times or until no more color is removed. Now shake with water; this removes the Orange 1 from the acetic ether. The water solution is freed from acetic ether by heating on the steam bath, and is then used to identify the color.

The water solution containing Ponceau 3 R, Indigo Disulfo Acid, and any Orange 1 not removed by the acetic ether is warmed on the steam bath until free from acetic ether. It is cooled, made up to 125 cubic centimeters with water, about 40 cubic centimeters of 25 per cent calcium chlorid solution added, and allowed to stand about 15 minutes. Ponceau 3 R and some Indigo Disulfo Acid are precipitated. Fifteen cubic centimeters of stannous chlorid solution is added and

stirred until all the blue color disappears. It should then be filtered immediately, as too long standing will destroy Ponceau 3 R. Ponceau 3 R remains on the filter paper. Wash with calcium chlorid solution until the blue that may have become reoxidized during filtration is washed out; dissolve in dilute ammonia solution; drive off the excess of ammonia, and test for Ponceau 3 R.

The filtrate, which should be practically colorless, is treated with hydrogen peroxid. If Indigo Disulfo Acid is present the solution will become deep blue in color.

The following table gives an outline of the above-described method of separation. It is desirable in carrying out this separation to follow the table, referring from time to time to the description included in this paper, as well as to the well-known reference works. Analysts who contemplate applying this method to the separation of mixtures of colors should first familiarize themselves with the method and with the behavior of the permitted colors by attempting the separation of mixtures of known permitted colors. It will probably be simpler to begin with two or three colors and finally to apply the method to a mixture containing all seven. Once this preliminary work has been done, the analyst will be able to detect the presence of interfering dyes. The presence of an unpermitted color will be indicated by the effect on the reactions of the permitted colors with standard reagents, and the unpermitted color may also interfere materially with the scheme of separation as outlined above.

While the method is not regarded as entirely free from defects, it has been found to give excellent results in the hands of men unskilled in color work, and it is described now with the hope that it may be of service to analysts who have been confronted with problems similar to those of the Bureau of Animal Industry.

Scheme for separating the seven permitted coal-tar colors.

Rub approximately 0.2 gram of sample in a mortar with supersaturated $(NH_4)_2SO_4$ solution; filter and wash with the $(NH_4)_2SO_4$ solution until washings are no longer colored red.

<table>
<tr><td colspan="2">Filtrate contains Amaranth and small amount Naphthol Yellow S. Shake with acetic ether.</td><td colspan="5">Residue contains Erythrosin, Naphthol Yellow S, Light Green S F Yellowish, Orange 1, Ponceau 3 R, and Indigo Disulfo Acid. Dissolve in H_2O, acidify with CH_3COOH, and shake with ether.</td></tr>
<tr><td>Acetic ether removes Naphthol Yellow S. This solution is discarded.</td><td>$(NH_4)_2SO_4$ solution contains Amaranth. Shake with acetone, which removes the Amaranth. Dilute with H_2O and drive off the acetone.</td><td>Ether contains Erythrosin. Wash with H_2O and then shake with dilute NH_4OH, which removes the Erythrosin. The excess of NH_3 is then driven off.</td><td colspan="4">H_2O solution is freed of ether and saturated with NaCl on steam bath, cooled, filtered, and washed with supersaturated NaCl solution until washings are colorless.</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">Filtrate contains Light Green S F Yellowish and Naphthol Yellow S. Shake with acetone. Separate acetone and salt solution.</td><td colspan="2">Precipitate is dissolved in H_2O and shaken three times with acetic ether.</td></tr>
<tr><td></td><td></td><td></td><td>Acetone removes Naphthol Yellow S. Wash with supersaturated NaCl solution; add H_2O to the acetone, and drive off the acetone on steam bath. Acidify with acetic acid; shake with amyl alcohol, which removes any traces of Orange 1 present, and then test the acidified water solution for Naphthol Yellow S.</td><td>NaCl solution contains Light Green S F Yellowish. Remove excess of salt by evaporating and taking up with alcohol; drive off alcohol and test for Light Green S F Yellowish.</td><td>Acetic ether contains Orange 1. Wash with supersaturated NaCl solution and then shake with H_2O, which takes out the Orange 1.</td><td colspan="2">The H_2O solution is warmed on steam bath until free from acetic ether, cooled, made up to 125 c. c. with H_2O, 40 c. c. of 25% $CaCl_2$ solution added, and allowed to stand about 15 minutes, 15 c. c. of $SnCl_2$ solution added and stirred until the blue color is destroyed. Filter.</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>Filtrate contains Indigo Disulfo Acid. It is treated with H_2O_2, which should give a deep blue color if Indigo Disulfo Acid is present.</td><td>The precipitate contains Ponceau 3 R. It is washed with $CaCl_2$ solution until free from blue color. Dissolve in dilute NH_4OH. The excess of NH_3 is then driven off.</td></tr>
<tr><td></td><td>Amaranth.</td><td>Erythrosin.</td><td>Naphthol Yellow S.</td><td>Light Green S F Yellowish.</td><td>Orange 1.</td><td>Indigo Disulfo Acid.</td><td>Ponceau 3 R.</td></tr>
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Approved:

JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, D. C., *June 5, 1911.*

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